

524 ANSWER 226 OF 368 CAPLUS COPYRIGHT 1997 ACS

AN 1994:509581 CAPLUS

DN 121:109581

TI Preparation of oligonucleotide monolayer

IN Debitsudo, Arubaguri

PA Mitsubishi Chem Ind, Japan

SO Jpn. Kokai Tokkyo Koho, 6 pp.

CODEN: JKXXAF

PI JP 06041183 A2 940215 Heisei

AI JP 92-196819 920723

DT Patent

LA Japanese

OS MARPAT 121:109581

AB In an oligonucleotide monolayer formed on a metal substrate surface, an oligonucleotide deriv. (I; R = H, thiol-protecting group; R1 = H, C1-3 alkyl; E = nucleic acid base; X = S, O; Y = H, OH; m = 1-20; n .gtoreq.8) is bonded to the metal substrate surface through the S atom. This oligonucleotide monolayer is suitable for a DNA sensor and as a material for mol. devices. Thus, I (R = R1 = Y = H, X = S, E = 9-adenyl, m = 4, n = 11) (II) was prepd. by the solid phase method using a Applied Biosystems oligonucleotide synthesizer. A Si wafer sequentially coated with 250.ANG. Cr and 15,000.ANG. Au vapor deposition films was dipped in a soln. of 0.05 mM II and 0.5 .mu.M dodecanethiol in EtOH for 24 h, pulled out from the soln., and washed with EtOH to give an oligonucleotide monolayer with thickness 19.ANG. and contact angle (H2O) 5.degree..

(19)日本国特許庁(JP)

(12)公開特許公報(A)

(11)特許出願公開番号

特開平6-41183

(43)公開日 平成6年(1994)2月15日

(51)Int.Cl. <sup>3</sup>	識別記号	庁内整理番号	FI	技術表示箇所
C 0 7 H 21/04 21/02	Z			
C 1 2 Q 1/68	Z	7823-4B		

審査請求 未請求 請求項の数2(全 6 頁)

(21)出願番号 特願平4-196819

(22)出願日 平成4年(1992)7月23日

(71)出願人 000005968

三菱化成株式会社

東京都千代田区丸の内二丁目5番2号

(72)発明者 デビッド アルバグリ

神奈川県横浜市緑区鶴志田町1000番地 三

菱化成株式会社総合研究所内

(74)代理人 弁理士 重野 剛

(54)【発明の名称】 オリゴヌクレオチド単分子膜

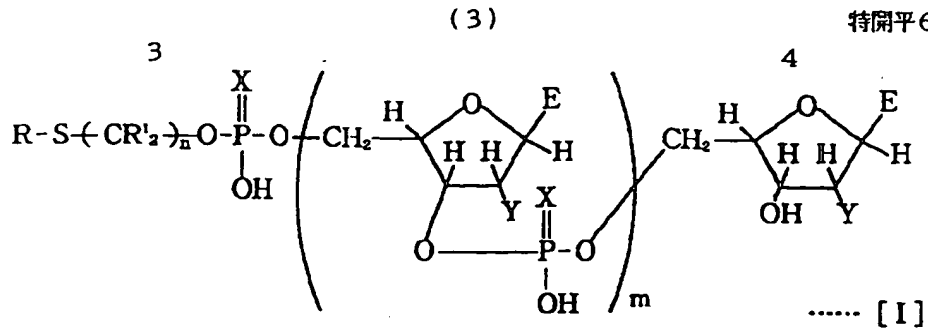
(57)【要約】

【目的】 DNAセンサーや分子素子等の機能材料の用途に好適な単分子膜を提供する。

【構成】 金属基板表面に形成された単分子膜であって、分子内にオリゴヌクレオチド構造を有する化合物が、硫黄原子を介して金属基板表面に結合している構造を有するオリゴヌクレオチド単分子膜。

【効果】 オリゴヌクレオチド構造を有する化合物よりなる単分子膜であれば、DNAセンサー、分子素子用材料として有用な単分子膜が提供される。





(式中、Rは水素又はチオールの保護基を示し、R'は水素又は炭素数1～3のアルキル基を示し、Eは核酸塩基を示し、XはS又はOを示し、Yは水素又はOHを示し、mは1～20の整数を示し、nは8以上の整数を示す。)

【0009】即ち、本発明者は、DANセンサーや分子素子等の機能材料の用途に好適な単分子膜を提供するべく、鋭意研究を重ねた結果、構造中にオリゴヌクレオチド構造を含む単分子膜はDNAセンサーや、分子素子等に用いるのに好適であることを見出し、本発明を達成した。

【0010】以下に本発明を詳細に説明する。

【0011】前記一般式(I)において、Eの核酸塩基としては、アデニン、グアニン、チミン及びシトシン等よりなる群から適宜選択される核酸塩基が挙げられる。

【0012】Rのチオールの保護基としては、アセチル\*

\*基、2-テトラヒドロピラニル基、又は、アルコキシ基等の置換基を有していても良いトリフェニルメチル基等が挙げられる。

【0013】nは8以上の整数であるが、nが大きすぎるもの、例えば20以上のものは試薬の入手が困難である。通常は、10～18であることが好ましい。

20 【0014】本発明に係るオリゴヌクレオチドのうち、Rが保護基であるものは、例えば次のプロセスに従って製造することができる。

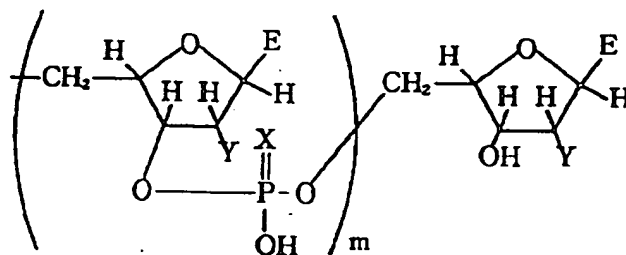
【0015】

【化4】



7  
 (A~Dの各ステップにおいて、R, R', X, n及びmは前記一般式 [I]  
 8  
 におけると同様である。

-(Kp)<sub>m</sub>-K- は一般式 [I] における

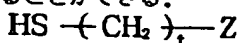


を表し、また、- Support は DNA 自動合成装置における、オリゴヌクレオチドの支持体を表す。

【0017】上記の各ステップのうち、Aのステップは、例えば、塩化メチレン等の溶媒中、ジイソプロピルアミン等の存在下に20~25℃の温度で行なわれる。また、B, C, Dの各ステップは、通常はDNA自動合成装置中に行なわれる。Bのステップは、例えば、アセトニトリル中で行なわれる。Cのステップは、XがSの場合は試薬としてテトラエチルチウラムジスルフィドを用いて行なわれ、XがOの場合は試薬としてヨウ素を用いて行なわれる。Dのステップはアンモニア等の塩基を用いて行なわれる。

【0018】また、前記一般式(I)において、Rが水素であるオリゴヌクレオチドは、上記で得られたオリゴヌクレオチドから常法により保護基を脱離させることにより得られる。

【0019】このようなオリゴヌクレオチドで構成される本発明の単分子膜は、前記一般式(I)において、RがHの場合は、前述のJ. Am. Chem. Soc., 111巻, 321~335頁(1989)記載の方法に準じた方法で製造することができる。また、前記一般式(I)においてRがチオールの保護基の場合には、次のようにして単分子膜を作成することができる。



(式中、tは8~18の整数、Zはアルキル基、ハロゲン原子、水素、シアノ基等を示す。)

【0023】

【作用】オリゴヌクレオチド構造を有する化合物よりなる単分子膜であれば、DNAセンサー、分子素子用材料として有用な単分子膜が提供される。

【0024】

【実施例】以下に実施例を挙げて本発明をより具体的に説明するが、本発明はその要旨を超えない限り、以下の実施例により限定されるものではない。

\*【0020】即ち、本発明に係る一般式(I)で表されるオリゴヌクレオチドをエタノール、エタノール/水(バッファー)、アセトニトリル/水(バッファー)等の溶媒に溶解し、この溶媒中にジクロル酢酸、メタンスルホン酸、p-トルエンスルホン酸ピリジン塩等の酸類を加え、溶液中でチオールの保護基を外し、チオールを取り出すか、或いは取り出して精製すること無く、その溶液中に清浄なAu, Ag又はCu等の重金属表面を有する基板を浸漬し、1時間~3日程度、10~50℃で放置した後、当該基板を引き上げることにより、該基板上に本発明の単分子膜を形成することができる。なお、この場合、フェノール、クレゾール等のフェノール類を保護基のアクセプターとして使用することができる。

30 【0021】また、本発明の単分子膜は、オリゴヌクレオチドと共に他のアルキルチオール誘導体を含む混合単分子膜として形成することもできる。ここで使用されるアルキルチオール誘導体としては、例えば、下記一般式(IX)で表されるアルキルチオール誘導体が挙げられる。

【0022】

【化6】



\*【0025】実施例1

前記一般式(I)において、R=H, R'=H, E=アデニン, X=S, Y=H, m=4, n=11であるオリゴヌクレオチド(Ia)の合成

A: 前記一般式(III)において、R=アセチル基, R'=H, n=11の化合物(IIIa)246mg、前記構造式(IV)の化合物355mg、及び、ジイソプロピルアミン386mgを塩化メチレン8mlに溶解し、20~25

※50

℃で1時間反応させた。

【0026】反応液を酢酸エチルで抽出し、抽出液をシリカゲルを担体とし、*n*-ヘキサン-クロロホルム-トリエチルアミン(4.5:4.5:1 容量比)を展開液とするカラムクロマトグラフィーにかけ、前記一般式(V)において、R=アセチル基、R<sup>1</sup>=H、n=11の化合物(Va)397mgを得た。このものの分析結果は次の通りである。

【0027】<sup>1</sup>H NMR (CDCl<sub>3</sub>)、TMS標準、300MHz

1.18 (m, 12H), 1.27 (m, 14H),  
1.58 (m, 4H), 2.32 (s, 3H), 2.64 (t of d, 2H), 2.86 (t, 2H),  
3.60 (m, 4H), 3.80 (m, 2H)

<sup>13</sup>C NMR (CDCl<sub>3</sub>), 75Hz

20.34, 24.58, 25.91, 28.78, 29.00, 29.12, 29.28, 29.42, 29.48, 29.52, 30.62, 31.19, 42.95, 58.29, 63.71, 117.66, 196.00

<sup>31</sup>P NMR (60% H<sub>3</sub>PO<sub>4</sub>, 外部) 109.25 Hz

-147.7

B~D: DNA合成装置中で反応を行なった。

【0028】DNA合成装置中で、前記一般式(VI)においてEがアデニンであり、YがHであり、mが4である化合物(VIa)に、上記化合物(Va)をアセトニトリル中で反応させて、前記一般式(VII)において、R=アセチル基、R<sup>1</sup>=H、n=11、m=4、Y=H、E=アデニンの化合物(VIIa)を得た。この化合物(VIIa)にテトラエチルチウラムジスルフィドを反応させて、前記一般式(VIII)において、R=アセチル基、R<sup>1</sup>=H、n=11、m=4、X=S、Y=H、E=アデニンの化合物(VIIIa)を合成した。この化合物(VIIIa)をアンモニア水で処理したところ、保護基のアセチル基がはずれ、目的とするオリゴヌクレオチド(Ia)を得た。

【0029】なお、これら一連の反応はApplied Biosystems User Bulletin 58-2 (1991)に記載の方法に準じて行なった。

【0030】得られたオリゴヌクレオチド(Ia)の高速液体クロマトグラフィーによる分析結果は次の通りである。

【0031】カラム C-18 逆相カラム  
グラジエント(直線)

A液: 0.05M酢酸アンモニウム

10 B液: アセトニトリル

グラジエントプログラム

スタート: A95%+B5%

30分後: A40%+B60%

37.66分後: A0%+B100%

検出波長 260nm

温度 25℃

上記の条件におけるリテンションタイムは20分であった。

【0032】単分子膜の製造

20 エタノールに上記で合成したオリゴヌクレオチド(Ia)

0.05mM及びドデカンチオール0.5μMを溶解

し、この混合溶液中に1.2cm×1.2cmのAu表面を有する基板(1.2cm×1.2cmのシリコンウェハー上にCrを膜厚250Å、更にその上にAuを膜厚15000Åの厚さに蒸着したものを)を25℃で24時間浸漬した。その後、基板を引き上げ、エタノールで洗浄し、本発明の単分子膜を得た。

【0033】得られた単分子膜の分析値は以下の通りである。

30 膜厚(エアソメトリーにて測定): 19Å

接触角(水): 6°

【0034】

【発明の効果】以上詳述した通り、本発明のオリゴヌクレオチド単分子膜によれば、DNAセンサー、分子素子用等の機能材料としての用途に工業的に極めて有用な単分子膜が提供される。



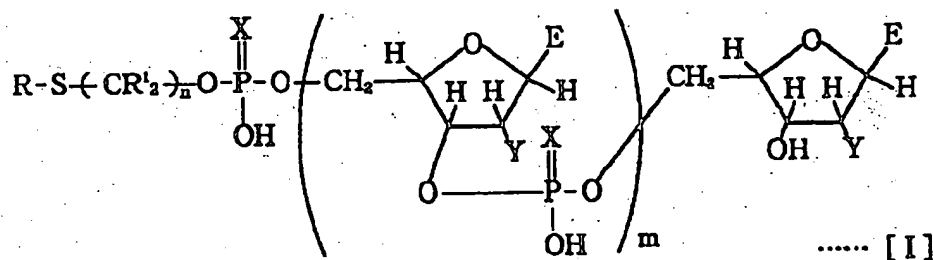


(Scope of Patent Claim)

**(Claim 1)** The subject is a monomolecular film formed on the surface of a metal substrate, and is an oligonucleotide monomolecular film having a structure in which a compound with an intramolecularly oligonucleotide structure is bonded to a metal substrate via sulfur atoms.

**(Claim 2)** The oligonucleotide monomolecular film referred to in Claim Item 1 is derived from the oligonucleotide shown in General Formula I below.

(Chemical Substance 1)



[Formula I caption:] (In this formula, R indicates the hydrogen or thiol protective group; R<sup>1</sup> indicates the hydrogen or carbon numbers 1-3 alkyl group; E indicates the nucleic acid base; X indicates S or O; Y indicates hydrogen or OH; m indicates the integers 1-20; n indicates integers of 8 and higher.)

[handwritten:] n=8

(Detailed Explanation of the Invention)

(0001)

Range of industrial use: The said invention concerns an oligonucleotide monomolecular film suitable for use as a DNA sensor and molecular device material.

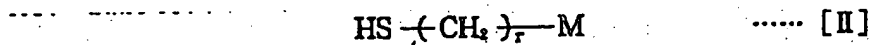
(0002)

Previous technology: There have already been reports regarding alkylthiol and monomolecular films of its derivatives. As an example of monomolecular films of alkylthiol derivatives, the substance shown in General Formula II below was reported as a component substance in J. Am. Chem. Society, Vol. 111, pp. 321-335 (1989).

[page 2, 2 of 3]

(0003)

(Chemical Substance 2)



[Formula 2 caption:] (In this formula, r indicates 8, 10, 11, 15, 17, or 21; M indicates -CH<sub>3</sub>, -CH = CH<sub>2</sub>, -COOH, halogen atom, -CO<sub>2</sub>CH<sub>3</sub>, or -CN.)

(0004)

Problem that the invention intends to solve: The alkylthiol derivative shown above in General Formula II did not have, as substitutional group M, a functional group that could be usefully employed as a DNA sensor and molecular device, etc. Accordingly, it would be difficult to say that monomolecular films composed of this alkylthiol derivative would be suitable for these uses.

(0005)

The objective of the said invention was, taking into account the previous circumstances enumerated above, to present a monomolecular film that would be composed of compounds that had an intramolecular oligonucleotide structure suitable for use as a functional material for DNA sensors and molecular devices, etc.

(0006)

Means for solving the problem: The oligonucleotide monomolecular film referred to in Claim Item 1 is characterized in that it is a monomolecular film formed on the surface of a metal substrate, and that it is an oligonucleotide monomolecular film having a structure in which a compound with an intramolecularly oligonucleotide structure is bonded to a metal substrate via sulfur atoms.

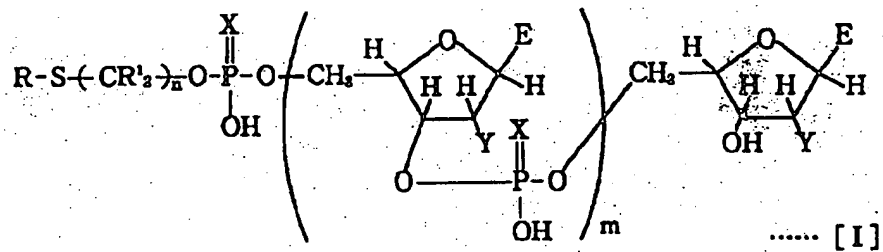
(0007)

The oligonucleotide monomolecular film referred to in Claim Item 2 is the monomolecular film referred to in Claim Item A, and has the characteristic that it is derived from the oligonucleotide shown in General Formula I below.

[page 2, 3 of 3]

(0008)

(Chemical Substance 3)



[page 3]

Patent Application Heisei 6-41183

[Formula I caption:] (In this formula, R indicates the hydrogen or thiol protective group; R<sup>1</sup> indicates the hydrogen or carbon numbers 1-3 alkyl group; E indicates the nucleic acid base; X indicates S or O; Y indicates hydrogen or OH; m indicates the integers 1-20; n indicates integers of 8 and higher.)

(0009)

As a result of diligent and repeated research, for the purpose of presenting the said invention as a monomolecular film that is suitable for use as a functional material for DAN [*sic*] sensors, molecular devices, etc., the inventor of the present invention found that a monomolecular substrate whose structure included an oligonucleotide structure was suitable for use as a DNA sensor, molecular device, etc., and the said invention was realized.

(0010)

The said invention is explained in detail below.

(0011)

The nucleic acid base indicated as E in General Formula 1 above is a nucleic acid base cited as an appropriate choice from among the group consisting of adenine, guanine, thymine, cytosine, etc.

(0012)

For the thiol protective group R, the triphenylmethyl group, etc. for which it is acceptable to have substitution groups such as the acetyl group, 2-tetrahydropyranyl group, or alkoxy group, is cited.

(0013)

Integers of 8 and higher are indicated above by n, but it is difficult to obtain reagents for values of n that are too high, such as 20 or higher. As a rule, a value of 10-18 is desirable.

(0014)

Among the oligonucleotides concerned with the said invention, those for which R is the protective group may, for example, be produced according to the following process.

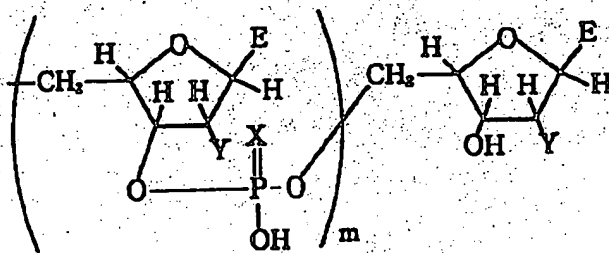
(0015)

(Chemical Substance 4)



(In each of the steps A-D, R, R', X, n and m are the same as in General Formula I above.

-(Kp)<sub>m</sub> K— appears in General Formula I as:



-Support indicates the oligonucleotide's support medium in the DNA automatic synthesizing mechanism.)

(0017)

Among the steps enumerated above, Step A is carried out at a temperature of 20-25° C, in a solvent such as methylene chloride, in the presence of diisopropylamine, etc. Steps B, C, and D are usually carried out in the DNA automatic synthesizing mechanism. Step B is, for example, carried out in acetonitrile. When X is S, Step C is carried out using tetraethylthiuram disulfide as the reagent; when X is O, iodine is used as the reagent. Step D is carried out using a base such as ammonia.

(0018)

In said General Formula I, Oligonucleotides for which the R is hydrogen are obtained, as a general rule, by inducing elimination of protective groups from oligonucleotides obtained as described above.

(0019)

When R is H in General Formula I above, the monomolecular film of the said invention, composed of these kinds of oligonucleotides, can be produced by a method based on the method published in the aforementioned J. Am. Chem. Society, Vol. 111, pp. 321-335 (1989). When R in General Formula I above is a thiol protective group, the monomolecular film can be produced as follows.

[page 5, 2 of 3]

(0020)

That is, dissolve the oligonucleotide shown in General Figure I for the invention in question in a solvent of ethanol, ethanol/water (buffer), acetonitrile/water (buffer), etc.; add acids such as dichloroacetic acid, metansulfonic acid, para-toluenesulfonic acid, pyridine salt, etc. to the solution, and remove the thiol protective group from the solution; without either extracting or extracting and purifying the thiol, immerse in the solution a substrate having a surface of heavy metal, such as pure gold, silver, or copper; leave it there for a period of 1 hour to 3 days, at 10-50° C, and then withdraw the said substrate; by this means the monomolecular film of the said invention can be made to form on the said substrate. In this case, phenols such as phenol, cresol, etc. can be used as acceptors of the protective groups.

(0021)

In addition, mixed monomolecular films can be formed, which include other alkylthiol derivatives together with the oligonucleotide for the monomolecular film of the said invention. For example, the alkylthiol derivative shown in General Formula IX below is cited as an alkylthiol derivative that can be used in this way.

(0022)

(Chemical Substance 6)



[Formula IX caption:] (In this formula, t indicates integers 8-18; Z indicates alkyl group, halogen atom, hydrogen, cyano group, etc.)

(0023)

Use: Monomolecular films are presented as useful for employment as materials for DNA sensors and molecular devices, if they are monomolecular films composed of compounds that have oligonucleotide structures.

[page 5, 3 of 3]

(0024)

(Embodiments) In the examples of implementation cited below, the invention in question will be more concretely explained. Insofar, however, as use does not substantially exceed the uses cited, the invention in question is not limited to the embodiments cited below.

(0025)

Embodiment 1: Synthesis of an oligonucleotide (Ia) such that R = H, R<sup>1</sup> = H, E = adenine, X = S, Y = H, m = 4, and n = 11 in General Formula I above

A: 246 mg of compound (IIIa) such that R = acetyl group, R<sup>1</sup> = H, and n = 11 in General Formula III above, 355 mg of the compound shown in Structural Formula IV above, and 386 mg of diisopropylamine were dissolved in 8 ml of methylene chloride, and allowed to react for 1 hour at 20-25° C.



(0026)

The reaction solution was extracted with ethyl acetate. Column chromatography was performed with the extracted solution as a carrier for silica gel, and with n-hexane-chloroform-triethylamine (in a capacity ratio of 4.5:4.5:1) as the developing solution. 397 mg of compound (Va) were obtained, such that R = acetyl group, R<sup>1</sup> = H, and n = 11 in General Formula V above. The results of analysis of this substance were as follows.

(0027)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), TMS normal, 300 MHz

1.18 (m, 12H), 1.27 (m, 14H), 1.58 (m, 4H), 2.32 (s, 3H), 2.64 (t of d, 2H), 2.86 (t, 2H), 3.60 (m, 4H), 3.80 (m, 2H)

<sup>13</sup>C NMR (CDCl<sub>3</sub>), 75 Hz

20.34, 24.58, 25.91, 28.78, 29.00, 29.12, 29.28, 29.42, 29.48, 29.52, 30.62, 31.19, 42.95, 58.29, 63.71, 117.66, 196.00.

<sup>31</sup>P NMR (60% H<sub>3</sub>PO<sub>4</sub>, exterior) 109.25 Hz

-147.7

B-D: Carried out reactions in the DNA synthesizing mechanism.

(0028)

In the DNA synthesizing mechanism, compound (Va) above was made to react in acetonitrile with compound (VIa) for which E was adenine, Y was H, and m was 4 in General Formula VI above. Compound (VIIa) was obtained, with R = acetyl group, R<sup>1</sup> = H, n = 11, m = 4, Y = H, and E = adenine in General Formula VII above. Tetraethylthiuram disulfide was made to react with this compound (VIIa), and compound (VIIIa) was synthesized such that R = acetyl group, R<sup>1</sup> = H, n = 11, m = 4, X = S, Y = H, and E = adenine in General Formula VIII above. When this compound (VIIIa) was treated with aqueous ammonia, the protective acetyl group was removed, and the

[page 6, 2 of 3]

oligonucleotide (Ia) was obtained which was the objective.

(0029)

This series of reactions was carried out according to the methods published in Applied Biosystems User Bulletin 58-2 (1991).

(0030)

The results of the analysis of the oligonucleotide (Ia) obtained using high performance liquid chromatography were as follows.

(0031)

Column            C-18                    Negative phase column gradient (line)

Solution A: 0.05 M ammonium acetate

Solution B: acetonitrile

Gradient Program

Start: A95% + B5%

After 30 minutes: A40% + B60%

After 37.66 minutes: A0% + B100%

Detected wavelength: 260 nm

Temperature: 25° C

The retention time under the above conditions was 20 minutes.

(0032)

[page 6, 3 of 3]

Creation of the monomolecular film

0.05 mM of the oligonucleotide (Ia) referred to above and 0.05 $\mu$ M of dodecanethiol were dissolved in ethanol. A 1.2 cm x 1.2 cm substrate with an Au surface (a 1.2 cm x 1.2 cm silicon wafer onto which a 250 Å coating of Cr, and on top of that a 15000 Å coating of Au had been deposited) was immersed in the combined solution for 24 hours at 25° C. After that, the substrate was removed, washed with ethanol, and the monomolecular film of the said invention was obtained.

(0033)

The results of analysis for the monomolecular film obtained are as follows.

Coating (Measured by ellipsometry): 19 Å

Contact angle (water): 6°

(0034)

Effectiveness of the invention: As indicated by the detailed account above, monomolecular films are presented as extremely useful industrially for use as DNA sensors and molecular devices, if they are oligonucleotide monomolecular films of the said invention.

